Experimental Cholera in Humans*

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Following on the isolation of the cholera vibrio by Koch in 1883, the aetiology of the disease was established in 1892 by the fulfilment of the third of "Koch's postulates" in the first recorded instance of intentional infection of human beings with cholera vibrios in the laboratory. In that historic experiment Pettenkofer and Emmerich both swallowed pure cultures of *Vibrio cholerae*. Pettenkofer manifested a mild diarrhoea, while Emmerich was reported to have developed severe cholera (Greig, 1929).

Since that time, and until recently, studies on the pathogenesis of the disease have been restricted to a variety of animals, which failed to reproduce, uniformly or in a typical manner, the cardinal manifestation of the disease, the voluminous outpouring of fluid.

Dutta and Habbu (1955) reported that the infant rabbit responded uniformly with choleraic diarrhoea after the introduction of living cholera vibrios into the intestinal tract at laparotomy. This was followed by the further demonstration that experimental cholera could be produced in infant rabbits by oral administration of multiple doses of sterile filtrates of lysates of heavy suspensions of cholera vibrios (Dutta *et al.*, 1959; Oza and Dutta, 1963) and even, in some cases, by sterile filtrates of the stool of cholera patients (Panse and Dutta, 1961). Thus it became evident, at least by such experiment, that choleraic disease could be produced in the absence of viable cholera vibrios. These demonstrations tended to revive the concept proposed by Koch (Pollitzer, 1959), that the signs and course of the disease in man could be explained on the assumption that the cholera vibrio produced a specific poison. While the pros and cons of this hypothesis have been argued by many cholera workers (Pollitzer, 1959), specific affirmative experimental evidence had been lacking.

Previous reports that products of cholera vibrios could cause death of experimental animals could not be construed as conclusive evidence for a role of "toxin" in the disease, since the characteristic symptomatology was not reproduced. Further support for the participation of a cholerigenous product released by cholera vibrios was provided by the observation (Finkelstein *et al.*, 1964; Finkelstein and Norris, 1964) that filtrates of broth cultures of cholera vibrios would produce experimental cholera in infant rabbits administered single doses of those products by the oral route. A highly potent antigenic cholerigenous product was produced in a simple, synthetic liquid medium supplemented with amino-acids. This filtrate, called Syncase, caused no symptoms when administered parenterally to infant rabbits. The pathological findings in the experimental cholera produced with cell-free filtrates were remarkably similar to those in humans. Survival of the infant rabbits with choleraic diarrhoea could be prolonged by administration of fluids according to their measured weight losses (R. A. Finkelstein, personal communication). It was most significant that the endotoxin of the cholera vibrio could not be implicated in the production of symptoms in the infant rabbit. Similarly, cholera endotoxin, administered orally in the form of comparatively large amounts of heat-killed cholera vibrios, was innocuous in human beings (Freter and Gangarosa, 1963).

Although in past years cholera has been responsible for hundreds of thousands of deaths annually, recent recognition of the fact that cholera represents a physiological derangement of fluid and electrolyte balance has led to the development of rational methods of therapy which can reduce the mortality of cholera practically to nil. In this hospital, during the severe cholera epidemic of 1959, only five fatalities occurred in a series of 134 bacteriologically confirmed cases of cholera. The deaths, which were associated with renal failure, all occurred in patients presenting severe haemoconcentration and shock on admission (Benyajati *et al.*, 1960). In more recent studies in Calcutta (Carpenter *et al.*, 1963) and in Dacca (Greenough *et al.*, 1964) some 140 patients have been treated with only two fatalities reported. The two deaths occurred in a 2-year-old child and in a man who had prior vascular collapse.

The present study was undertaken to determine whether the cholerigenous factors which produce experimental cholera in the laboratory animal would also elicit choleraic manifestations in human beings. Primary considerations were that the administration of cell-free non-replicating products could be expected to produce a self-limited syndrome, less severe and shorter in duration than the disease produced by active infection, and that these materials administered into the digestive tract would be unlikely to produce untoward systemic effects other than the manifestations of choleraic diarrhoea, which can effectively be treated by fluid and electrolyte replacement, especially under controlled conditions when treatment could be given promptly at the onset of symptoms.

Materials and Methods

Syncase cholerigen was prepared, *V. cholerae* strain 569 B Inaba being used according to the method described by Finkelstein *et al.* (1964). Essentially, the product was a sterile filtrate of a 20-hour aerated broth culture of the organism in the carbohydrate-salts medium supplemented with casamino acids.

The two volunteers used in the study were selected on the basis of their willingness to participate, their healthy condition, and the fact that they had no previous history of cholera or cholera immunization. Both subjects had indicated that their bowel movements were normal during the three to four months prior to the experiment.

Preparations were made for any anticipated emergencies. There was at least one physician by the side of the subject at all times. Blood-pressure and pulse rate were recorded frequently. Serum and faecal volumes were recorded by means of graduated containers. Water and electrolytes, consisting of NaCl, KCl, and $NaHCO_3$, were replaced according to the amount lost.

To avoid possible inactivation of the cholerigenous factors in the stomach, the materials were introduced into the small intestine by means of the intestinal biopsy capsule described by Crosby and Kugler (1957). The capsule was swallowed the

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night before the experiment to allow it to pass into the small intestine. Position of the capsule was checked by *x*-ray examination and by withdrawal of bile stained by fluids. Cholerigen was given by means of a syringe attached to the polyethylene tubing connected to the capsule. The capsule was located in the duodenum of the first subject and in the jejunum of the second.

The first subject received the cholerigen gradually in 10-ml. amounts at intervals of 10 minutes until a total dose of 100 ml. had been given. The second subject received the cholerigen in 25-ml. amounts gradually at 15-minute intervals to the total dose of 400 ml. These amounts correspond approximately, on a weight-equivalent basis, to one-fifth and four-fifths of a cholerigenous dose for the infant rabbit. Rectal swabs and stool samples were cultured for the presence of enteric pathogens and cholera vibrios at repeated intervals before and after administration of the cholerigen.

Results

The first subject passed only one loose stool 12 hours after administration of the cholerigen. Otherwise, he had no other symptoms or manifestations. No enteric pathogens could be isolated.

The second subject, who received four times the dosage as the first, started having loose stools, some nausea, and vomiting one and a half hours after administration of the cholerigen was completed (approximately three and a half hours after administration was started). He was then placed in the cholera cot. Two hours later his stools had increased in frequency and had become watery: the *p*H was 6.92, sodium 73 mEq/l., and potassium 10.6 mEq/l. The colour remained dark brown. The urine was normal. When the specific gravity of the whole blood had risen to 1063 fluid replacement was initiated, and was continued until the diarrhoea stopped. Blood-pressure and pulse rate remained normal. The nausea and vomiting subsided about 10 hours after onset. The subject had no abdominal pain, but his gurgling sound was increased, and he complained only of abdominal distress and the increased frequency of stools. No signs of dehydration or shock appeared at any time. The diarrhoea stopped about 48 hours after onset. The peak of the diarrhoea was in the first 24 hours. The total stool volume was 14,800 ml. and the total volume of fluid replacement was 15,120 ml. The subject was able to get up and walk after replacement fluids were discontinued, and, except for the inconvenience of the catheters, could have done so during the early episode. Serum electrolytes and other laboratory findings are presented in Tables I and II. No enteric pathogens or cholera vibrios could be isolated from the multiple specimens that were taken.

TABLE I.—*Blood Count*

Period	Hb (mg/100 ml.)	R.B.C.	W.B.C.	Differential Count			
				P	L	E	M
Control	13·0		7,150	75	23	1	
During diarrhoea ..	11·8	5·51	9,450	84	16		
Convalescence	12·8	4·81	7,100	72	25	2	1

TABLE II.—*Fluid and Electrolyte Data*

No. of Hours After Onset	Wt. (kg.)	Fluid Intake (ml.)			Fluid Lost (ml.)			Electrolytes (mEq/l.)				Sp. gr. Whole Blood
		Parenteral	Oral	Total	Urine	Stool	Total	Na	K	Cl	CO_2	
Control	52							140	4·9	103	25	1060
6	52	4,000	1,100	5,100	50	3,700	3,750	140	5·1	103		1063
30		7,060	1,400	8,460	1,050	9,200	10,250	138	4·9	95·3	18·2	1061
54		2,040	850	2,890	1,000	1,900	2,900					
78		2,020	1,600	3,620	1,450		1,450					
102	50		1,000	1,000	1,300		1,300					
Total		15,120	5,950	21,070	4,850	14,800	19,650					

Discussion

In the present study an experimental disease resembling cholera was produced in a human volunteer who received a sterile filtrate, Syncase (Finkelstein *et al.*, 1964), containing products of the cholera vibrio released during growth in a simple chemically defined medium supplemented with casamino acids. The study thus extends to the human the observations of Dutta and his colleagues and of Finkelstein *et al.* that experimental cholera may be produced in infant rabbits in the absence of viable cholera vibrios. The observation that only a transitory diarrhoea was produced in the first subject who received a smaller dose of the Syncase cholerigen suggests that the severity of the symptoms is related to the dosage administered. Fortuitously, it appears that the minimal dose for a detectable response and a dose which produced a maximal desirable effect may have been defined by the use of two subjects.

These observations tend to support the validity of the infant rabbit for studies of the pathogenesis of cholera. It may now be expected that further studies in the infant rabbit may yield important basic information regarding the locus and mode of action of the cholerigen which may be directly applicable to the human disease. Certain aspects of the disease, however, such as the effect of the nutritional status of the host, the relationship of pre-existing intestinal abnormalities (Sprinz *et al.*, 1962) to susceptibility, the effects of immunization or of recovery to cholera on resistance, and further definition of the physiological and metabolic lesions of cholera must ultimately be resolved in the studies with human subjects. The present study suggests that many of these aspects may be investigated with minimal risk by further study under controlled conditions in human volunteers with the use of sterile cholerigen.

The observations presented herein also raise the question of whether a "sterile" food-borne choleraic disease may exist in nature as a result of preformed cholerigen in infected foodstuffs or water which have become disinfected by phage action of other conditions deleterious to the cholera vibrio (Finkelstein, personal communication). Some evidence for this hypothesis is suggested by observations (Benyajati, in preparation; Carpenter *et al.*, 1963; Greenough *et al.*, 1964) that in cholera patients treated with antibiotics diarrhoea persists for 24 hours or more after negative culture.

Summary and Conclusions

Experimental cholera has been produced in a human subject after introduction of a sterile filtrate of a culture of ***Vibrio cholerae*** into the small intestine by means of the Crosby capsule. The results lend support to the view that the infant rabbit is a valid tool for the study of the pathogenesis of cholera, and suggest that Syncase cholerigen may indeed represent the factor responsible for the diarrhoea of cholera in humans.

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Physical Properties of Hydrophilic Gel Contact Lenses

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Recent development of hydrophilic synthetic polymers to compete with conventional methyl methacrylate (Perspex, Lucite, Plexiglas) in the manufacture of contact lenses has created the need to evaluate the physiologically important properties of these two materials. Some unpublished (or published only in the public press) clinical investigations in Czechoslovakia, where the hydrophilic lens originated, claim that contact lenses made of this material may be tolerated on the eye longer than the conventional methyl methacrylate lens. High oxygen transmissibility and high water permeability are the physical properties which would lead to this important advantage of the hydrophilic polymer over the conventional polymer. We have measured these properties of hydrophilic and conventional methyl methacrylate lenses and report them here.

It is generally recognized that the oxygen necessary to maintain the metabolism of the cornea is brought to the epithelial surface under a corneal contact lens by air-saturated tear fluid flowing under the lens. Each blink rocks the lens slightly and thereby pumps fresh tear fluid to the corneal surface. The hydrophilic lens is much softer and more pliable than conventional methyl methacrylate and is observed clinically to mould itself to the cornea. For the hydrophilic lens, therefore, high oxygen transmissibility or high water permeability is essential if the hydrophilic lens is to allow passage of oxygen from the air to the cornea. Our measurements, described below, indicate that oxygen transmissibility and water permeability of the hydrophilic material are not appreciably greater than those of conventional methyl methacrylate.

Methods

Oxygen transmissibility was measured by passing nitrogen gas over a lens, sealed by heavy grease to a small air-filled chamber, about 3.7 c.mm., which was in turn sealed to the surface of a Clark-type membrane-covered oxygen electrode. The rate of decrease of oxygen tension in the chamber, together with the dimensions of the chamber and lens, was used to calculate transmissibility. No attempt was made to obtain precise data; instead, emphasis was put on the comparison of hydrophilic and conventional methyl methacrylate lenses.

Water permeability was measured by noting the rate at which water flowed through a water-submerged lens when it was held by vacuum against the end of a clear plastic rod with an axial hole 1 mm. in diameter. The end of the rod had a curved surface of 5 mm. radius so that the lens would hold against the surface and allow no water-passage except through the lens material itself.

Results

The oxygen transmissibility of both hydrophilic and conventional methyl methacrylate is no higher than 4×10^{-12} sq. cm. O_2 (S.T.P.)/sec./mm. Hg. The water permeability of both materials is no higher than 1.4×10^{-14} cm.^{4} sec./dyne.

Discussion

The corneal epithelium requires about 6 μl. of oxygen per hour per square centimetre of surface. This oxygen must be provided by the air, through the tear layer. Oxygen in the aqueous humour cannot supply the epithelium because of the resistance to oxygen transport offered by the stroma. If it is assumed that the epithelium can create the maximum oxygen tension gradient across the contact lens, 155 mm. Hg, then only about 0.05 μl. of oxygen per sq. cm. per hour will be obtained. This is about 1/100 of the necessary oxygen. It is apparent, then, that neither the hydrophilic nor the conventional methyl methacrylate lens can supply oxygen to the cornea by oxygen passage through the lens material.

The water permeability of both lens materials is no greater than that of the epithelium. But, since a lens is usually ten times thicker than the epithelium, resistance to water-flow across the lens will be ten times that across the epithelium. No process can be visualized that would deliver a useful amount of tear fluid through either the hydrophilic or the conventional lens material.

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